

NTP Research Concept: Dong Quai

Project Leader:

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Nomination Background and Rationale:

Dong quai was nominated for comprehensive toxicological characterization by a private individual based on its widespread use as a dietary supplement, suspicion of toxicity based on estrogenic activity, and lack of adequate toxicity data. Additionally, there is uncertainty for the potential adverse effects in pregnant or nursing mothers and in individuals taking non-steroidal anti-inflammatory drugs. In traditional Chinese medicine, dong quai is the second-ranked ingredient. As such, it is used in numerous herbal combinations for a broad array of ailments. In the United States, dong quai is marketed as a dietary supplement with purported effects that include the maintenance and balance of sex hormones, the treatment of menstrual irregularities and menopausal symptoms, and as a general tonic for the female reproductive system. To a lesser extent, exposure may occur through use of cosmetics containing dong quai as an ingredient.

There are no subchronic or chronic toxicology or carcinogenicity studies available in the literature for dong quai or its primary constituents. Many of the studies that are in the literature were conducted with either formulations of herbals that contain dong quai or with specific constituents found in the root, and not with dong quai alone. Dong quai root contains an abundant number of constituents including alkyl phthalides, furanocoumarin, coumarins, terpenes, phytosterols, organic acids, and an immune-stimulating polysaccharide. The major constituents are considered to be ligustilide, ferulic acid, and 3-butylidenephthalide. Additionally, it has been noted that the carcinogen safrole is also present in the essential oil of dong quai.

There are conflicting results in the literature on the estrogenic effects of dong quai. Some studies indicate that dong quai produces estrogen-like effects including weak binding to the estrogen receptor (ER), ER-mediated increases in cellular proliferation, and the stimulation of estrogenic responses in ovariectomized rats. Other similar studies have failed to produce these estrogenic effects. One study even demonstrated that ethanol extracts of dong quai elicited anti-estrogenic and anti-androgenic activity.

Dong quai extracts can modify the activity of a variety of enzymes. Aqueous and ethanol extracts increase hepatic microsomal protein content and decrease cytochrome P450 levels. Depending on the extract, differential modulation of P450 isoform activities have been demonstrated, including increased or decreased activity for CYP3A, which is primarily responsible for the metabolism of a large number of environmental chemicals and pharmaceuticals. Therefore, there is the potential for dong quai to interact with drugs, chemicals, and other dietary supplements, especially estrogenic drugs used for birth control, hormone replacement, and selective estrogen receptor modulators due to both the potential estrogenicity of dong quai and the effects on metabolizing enzymes. Additionally, dong quai may exacerbate or potentiate the effects produced by

pharmaceuticals or dietary supplements that affect heart rhythm, alter blood clotting, or induce photosensitivity. In fact, some studies suggest that particular components of dong quai alone may induce phototoxicity.

Studies have demonstrated that dong quai, some of its specific constituents, or mixtures of herbal compounds that contain dong quai had cytotoxic, antiproliferative, and proapoptotic effects in numerous human and rodent cancer cells. Conversely, polysaccharides from dong quai increased proliferation in highly proliferative hematopoietic and gastrointestinal mucosal cells, and provided protection against the cytostatic effect of anticancer drugs on these cells.

Many human studies evaluating the beneficial effects of Dong quai have been conducted to date; many focused on the alleviation of menopausal symptoms. Overall, these studies suggest that there is little evidence supporting beneficial effects of dong quai for any condition, including menopausal symptoms, kidney diseases, and coronary artery disease. It is of interest to note that although dong quai is considered the “female ginseng” and serves as an all-purpose herb for women, a recently completed (unpublished) clinical trial is also investigating the effects of dong quai on hot flashes in men with prostate cancer.

Dong quai and its components have also been shown to alter gene expression in various tissues and cell types; modulate cell proliferation; interact with histamine H₁, 5-HT_{1A}, 5-HT₇, and γ -aminobutyric acid receptors; and modulate the immune system. Additional activities ascribed to dong quai and its active constituents include anxiolytic, insecticidal, and antifungal activity.

Key Issues:

Dong quai is commercially available in a variety of forms as a dietary supplement, including fluid extracts, tinctures, decoctions, capsules, tablets, and its essential oil as well as dried leaf preparations. It is available solely, but also in a mixture with other herbs, vitamins, or minerals. Methods of processing the dong quai root includes soaking, steaming, boiling, or frying in Chinese wine, rice vinegar, juice of ginger or other herbs. Analytical studies have demonstrated that there are notable differences in the constituents of dong quai based on the processing method. Differences in the constituent profile based on processing techniques could significantly impact the toxicological response. Therefore, serious consideration needs to be given to the specific formulation of Dong Quai selected for study.

Herbs grown in China may be harvested from contaminated soils, which may lead to contamination by heavy metals or pesticides. Some studies have identified significant lead concentrations in dong quai preparations. Additionally, there is concern for bacterial contamination. A previous NTP *Salmonella* assay was insufficient due to a contaminated sample of dong quai. Toxicosis has been reported when mildewed dong quai was given orally.

Proposed Approach:

The overall goal of this project is to characterize the toxicity and carcinogenicity of dong quai following oral exposure in male and female rats and mice. Based on the purported effects, this is a dietary supplement that is primarily taken by women of reproductive age, but potentially men, as well. An essential aspect of this project is to investigate the teratogenic potential of dong quai and its effect on reproduction and sexual maturation. However, since there appears to be little standardization of dong quai preparations in the market with respect to concentrations of its components, the selection of a specific test article is a critical issue given that the variability between preparations of dong quai may elicit differences in toxic responses. Therefore, a tiered approach will be applied in which the critical issue of investigating the potential impact of dong quai preparation selection is addressed first, prior to moving forward with additional toxicology studies.

Specific Aims

Specific Aim 1. Investigate potential differences in toxicological response between various preparations of dong quai in short-term *in vivo* and *in vitro* assays including the Hershberger and uterotrophic assays, and nuclear receptor assays. These studies will provide critical data for the determination of test material selection, and serve to further characterize toxicity and identify target tissues.

Specific Aim 2. Conduct oral 14-day subacute, 90-day subchronic, and 2-year chronic studies in male and female rats and mice to characterize the toxicity and carcinogenicity of dong quai. Additionally, re-evaluate dong quai for mutagenicity in *Salmonella* assay, and conduct an *in vivo* micronucleus assay.

Specific Aim 3. Conduct reproductive and developmental toxicity studies with special emphasis on landmarks of sexual maturation to investigate the biological implications of potential alterations of sex hormones and interactions with sex hormone receptors.

Specific Aim 4. Conduct studies to evaluate the immunotoxicity and phototoxicity of dong quai, including short-term studies to identify and quantitate photo-induced DNA adducts.

Significance and Expected Outcome:

Dong Quai is included in the list of food ingredients that are generally recognized as safe for their intended use, within the meaning of Section 409 of the Federal Food, Drug, and Cosmetic Act (21CFR182.10). Like most herbal remedies and dietary supplements, information on the extent of exposure is not readily available. However, very limited information exists on the toxicity of dong quai. These proposed studies would address the lack of toxicity data for dong quai and provide much-needed information on the safety of dong quai for the FDA and the public.